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Correlated responses of fatty acid composition, grain quality and agronomic traits to nine cycles of recurrent selection for increased oil content in oat

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Summary

Increases in the groat-oil content of oat (*Avena sativa* L.) increase the energy value of the grain and improve the feasibility of extracting oat oil for use as a vegetable oil. Nine cycles of recurrent selection for greater groat-oil content conducted in a genetically broad-based oat population resulted in dramatic increases in groat-oil content. Our objectives were to determine if selection for greater groat-oil content affected fatty acid composition, grain quality traits (test weight and seed weight), or agronomic traits (straw yield, biomass, harvest index, heading date, and height). We evaluated 100 random lines from the base (C0) population and each of the nine selection cycle populations in three environments in order to estimate means, genetic variances, heritabilities, and genotypic and phenotypic correlations of grain quality and agronomic traits. We also evaluated 20 random lines from each population to estimate changes in fatty acid contents. Oleate and stearate contents increased over cycles of selection, as did the ratio of unsaturated to saturated fatty acids. Palmitate, linoleate, and linolenate contents and all grain quality and agronomic traits except harvest index decreased over cycles of selection. There was no evidence for reduced genetic variance or heritability in C9 for any trait, but the genotypic and phenotypic correlations between agronomic traits and oil content fluctuated over cycles. Selection for increased groat-oil content improved oil quality but reduced grain quality and agronomic performance of the population.

Abbreviations: NMR – nuclear magnetic resonance; REML – restricted maximum likelihood; U:S – ratio of poly- and monounsaturated fatty acid content to saturated fatty acid content; 16:0 – palmitate, 18:0 – stearate, 18:1 – oleate, 18:2 – linoleate, 18:3 – linolenate

Introduction

Increases in the groat (caryopsis)-oil content of oat grain are desirable for improving the feeding value of oat grain for livestock and for developing oat as an oilseed crop. Oat grain is a high quality animal feed, with a desirable amino acid profile, but has low metabolizable energy, primarily because of the high proportion of fibrous hull (Cuddeford, 1995). Increasing grain-oil content and reducing hull percentage are two ways in which the nutritive value of whole oat grain can be improved. Oil extracted from oat groats is suitable for human consumption, with good flavor and stability (Kalbasi-Ashtari & Hammond, 1977). The development of markets for oat oil and defatted oat flour would enhance the economic value of the oat crop (Kaaria, 1990). The economic feasibility of using oat as an oilseed crop, however, depends on the availability of oat cultivars with groat-oil contents in the range of

17% with acceptable agronomic performance (Frey & Hammond, 1975).

Frey & Holland (1999) recently reported the development of an oat population from nine cycles of recurrent selection for higher groat-oil content. The population resulting from nine cycles of selection (C9) had higher mean oil content and oil yield than any previous oat population reported (Frey & Holland, 1999). Numerous genotypes were identified in the C9 population that had oil contents greater than 17%, and one line had an oil content of more than 18% (Frey & Holland, 1999). The oil content of some lines from the C9 population meet the criteria proposed by Frey & Hammond (1975) required for oat to be an economically viable oilseed crop. The development of high-oil oat cultivars from this population depends on recovering genotypes with a combination of high oil content, good oil quality, and good grain quality and agronomic characteristics.

The fatty acid composition of oat oil is the primary determinant of oil quality for human consumption because of its effects on human health, particularly on cholesterol levels. Saturated fatty acids, including palmitate (16:0), but excluding stearate (18:0), contribute to higher serum cholesterol levels in humans (Lichtenstein et al., 1998). A diet low in saturated fatty acids, *trans* fatty acids, and cholesterol and relatively high in unsaturated fatty acids is recommended for humans (Lichtenstein et al., 1998). Polyunsaturated fatty acids, however, are susceptible to oxidation, leading to rancidity and undesirable flavors in oils (Forsberg & Reeves, 1992) and in stored oat grain products (Karow & Forsberg, 1984). High levels of linolenate (18:3) are particularly undesirable because 18:3 oxidizes easily (Karow & Forsberg, 1984). Therefore, the quality of oat oil for human consumption could be improved by lowering palmitate and linolenate contents, and by increasing oleate and linoleate contents. Forsberg & Reeves (1992), however, suggested that linoleate is rarely limiting in human diets, and that reductions in linoleate content could reduce problems of rancidity associated with storage of oat grain without significantly reducing the dietary quality of oat grain products.

Schipper et al. (1991) reported that, after six cycles of recurrent selection for increased oil content in oat, concentrations of palmitate and linolenate decreased moderately, linoleate (18:2) content decreased greatly, stearate content increased moderately, and oleate (18:1) content increased greatly. They observed that the ratio of unsaturated fatty acids to saturated fatty acids increased over cycles of selection, and that significant genetic variation still existed for each fatty acid content except linolenate content. These results suggested that continued selection beyond cycle six for higher groat-oil content would cause further changes in the fatty acid composition.

High-oil oat cultivars must have adequate grain quality and agronomic performance for use as either an oilseed or high-energy grain feed crop. Important grain quality traits include test weight, seed weight, and groat fraction. Test weight is the most generally used indicator of grain quality in oat (Forsberg & Reeves, 1992), groat fraction largely determines feeding value of the grain (Cuddeford, 1995), and light weight kernels are undesirable for milling (Burnette et al., 1992). Important agronomic traits for oat cultivars include greater grain and straw yields, lower plant height (to reduce lodging), and adaptive flowering time (Holland, 1997).

Branson & Frey (1989a) reported that the agronomic characteristics, including grain yield, test weight, groat fraction, and seed weight, of the C0 through C3 populations did not differ. They suggested that changes in oil content could be achieved without unfavorable correlated responses in agronomic traits in this population. Schipper & Frey (1992b), however, reported that unfavorable correlated responses in key agronomic traits were observed after six cycles of recurrent selection in this population. Later cycles of selection tended to have lower biomass, grain yield, and test weight (Schipper & Frey, 1992b). The increased bioenergy required to produce higher amounts of oil likely resulted in less energy being used for carbohydrate production, and contributed to the decreases observed in grain yield (Schipper & Frey, 1992a). Frey & Holland (1999) reported that the traits that directly affect oil yield (groat-oil content, grain yield, and groat fraction) responded differently to nine cycles of recurrent selection for higher oil content. Groat fraction did not change, and increases in groat-oil content more than offset decreases in grain yield, resulting in steady increases in oil yield with each cycle of selection. Experimental lines with elevated groat-oil contents exhibited some serious agronomic deficiencies, including lower grain yields and excessive lodging and disease susceptibility, in multi-environment yield trials (Frey & Holland, 1999).

The objectives of this study were to determine (1) the correlated responses of fatty acid composition of groat-oil, grain quality, and agronomic characteristics of this oat population to nine cycles of recurrent se-

lection, and (2) if genetic variation for grain quality and agronomic traits or genetic correlations between these traits and groat-oil content have been affected by selection. The results of this study will guide future efforts at developing oat cultivars with elevated groat-oil content, good oil quality, and acceptable agronomic and grain quality characteristics via multi-trait recurrent selection schemes.

Materials and methods

Population development and recurrent selection method

The development of the base population was described in detail by Branson & Frey (1989b). Briefly, eight interspecific F_1's (from matings of eight high-oil *A. sterilis* accessions and eight high-oil *A. sativa* cultivars) were crossed to eight locally adapted cultivars. F_2 seeds from each three-way mating were space-planted in the field and culled for cultivated type, adapted maturity, and plant height. Groat-oil contents of the remaining plants were assayed using the wide-line nuclear magnetic resonance (NMR) method described by Conway & Earle (1963). Selected F_2-derived lines were intermated, and the same criteria were used to select among F_2 progeny from these matings. Selected F_2-derived lines were mated at random to five unrelated locally-adapted cultivars, the F_1 plants of these crosses were intermated, and the S_0 plants from these crosses formed the base (C0) population for selection. The recurrent phenotypic selection scheme was described by Branson & Frey (1989b) and the development of C0 through C9 populations was described by Frey & Holland (1999).

Evaluation of grain guality and agronomic traits

One hundred S_0-derived lines were randomly chosen from each cycle population (C0-C9) for the evaluation experiment. The generation of seeds within each line varied among cycles: $S_{0:4}$ lines represented populations C0 through C2, $S_{0:3}$ lines represented C3 through C5, $S_{0:2}$ lines represented C6 through C8, and $S_{0:1}$ lines represented C9. Five check cultivars ('Dal', 'Hamilton', 'Ogle', 'Starter', and 'Webster') were included as repeated entries four times within each replication. The entries were planted in randomized complete block experiments with two replications at three Iowa locations (Ames, Kanawha, and Nashua) in 1992. Plots were hills of 30 seeds each, planted on a grid and spaced 0.3 m in perpendicular directions. Two rows of hills of a common cultivar were planted as a border around each experiment to provide competition for peripheral plots. Experiments were treated with the systemic fungicide 1-(4-chlorophenoxy)-3,3-dienethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone to prevent crown rust disease (incited by *Puccinia coronata* Corda var. *avenae* W.P. Fraser & Ledingham). Grain yield, straw yield, above-ground biomass, and harvest index were recorded on every hill plot. Heading date and plant height from ground level to the tips of the panicles were measured on every plot at Ames only. After threshing, seeds of the same entry from the two replicate hill plots at a location were combined to provide sufficient seed for measuring test weight, groat fraction, and weight of 100 seeds. A sample of seeds from each entry-by-location combination was dehulled to provide 3.5 to 5.0 g of oven-dried groats that were assayed for oil content using NMR by Dr Alexander at the University of Illinois.

Statistical analysis of grain quality and agronomic characteristics

Means of cycle populations and checks and standard errors for their comparisons were obtained from a mixed models analysis, considering genotypes, replications, and locations (where appropriate) to be random effects and cycles to be fixed effects, and considering the checks to be a separate cycle population. A regression model including both linear and quadratic responses of population means (excluding checks) to cycles of selection was developed for each trait. If the quadratic term was significant at $p < 0.05$, then both regression parameters were reported, and the mean response per cycle was estimated as the difference between C9 and C0 population means, divided by nine. If the quadratic term was not significant, then it was dropped, and a reduced model including only the intercept and the linear regression parameter was developed. In this case, the linear regression estimate from the reduced model was reported and this regression estimate also served as the estimate of the mean response per cycle. All analyses were conducted using SAS Proc MIXED (SAS Institute Inc., 1997).

Genotypic variance components and their standard errors for each trait and cycle combination were estimated separately using the restricted maximum likelihood method in SAS Proc MIXED, considering all effects in the model except the intercept to be random effects. Heritability on a plot-basis was estimated

for straw yield, biomass, harvest index, heading date, and height as:

$$\hat{H}_{plot-basis} = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \hat{\sigma}_{GE}^2 + \hat{\sigma}_g^2}.$$

Heritability on a bulked-sample-basis was estimated for test weight and 100-seed weight, because these traits were measured on samples of seed bulked from two replicate plots per location rather than individual plots. In this case, experimental error among bulked samples within a location was not estimated, and genotype-by-environment interaction (confounded with experimental error) was the residual variance from the analysis over locations of bulked sample values. Heritability on a bulked-sample-basis was calculated as:

$$\hat{H}_{bulked-sample-basis} = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \hat{\sigma}_{GE}^2}.$$

Approximate standard errors for heritabilities were estimated using the delta method (Lynch & Walsh, 1997).

Genotypic and genotypic-by-environmental covariance components between each trait and groat-oil content were estimated using restricted maximum likelihood (REML) in SAS Proc MIXED. Multivariate REML estimates were obtained with Proc MIXED by treating each pair of variables as repeated measurements of a single variable at each location (Wright, 1998). Groat-oil content was measured on samples bulked across replications at each location, therefore, the phenotypic correlation was estimated on the basis of genotype-environment combination means, rather than on a plot-basis. Genotype means at each location for straw yield, biomass, harvest index, heading date, and height were estimated. Data sets were created containing each pair of traits for which correlation analysis was desired. In these data sets, a new classification variable was created to indicate the name of the trait, and a single response variable was created corresponding to the trait indicated by the classification variable. A multivariate analysis was then performed on each cycle independently, following Wright (1998). This analysis produced REML estimates of the genotypic variance and covariance components and of the genotype-by-environment variance and covariance components. The genetic correlation was estimated as:

$$\hat{r}_{g12} = \frac{\hat{\sigma}_{G12}}{\hat{\sigma}_{G1}\hat{\sigma}_{G2}}.$$

where $\hat{\sigma}_{G12}$ is the estimated genotypic covariance between traits 1 and 2 and $\hat{\sigma}_{Gi}$ is the estimated genotypic standard deviation for trait i (i = 1 or 2). The phenotypic correlation on a location-mean basis was estimated as:

$$\hat{r}_{p12} = \frac{\hat{\sigma}_{P12}}{\hat{\sigma}_{P1}\hat{\sigma}_{P2}} = \frac{\hat{\sigma}_{G12} + \hat{\sigma}_{GE12}}{\sqrt{\hat{\sigma}_{G1}^2 + \hat{\sigma}_{GE1}^2}\sqrt{\hat{\sigma}_{G2}^2 + \hat{\sigma}_{GE2}^2}}$$

where $\hat{\sigma}_{P12}$ and $\hat{\sigma}_{GE12}$ are the phenotypic covariance and genotype-by-environment covariance, respectively, between traits 1 and 2, and $\hat{\sigma}_{Pi}$ and $\hat{\sigma}_{GEi}$ are the phenotypic and genotype-by-environment standard deviations for trait i. Approximate sampling variances for the genotypic and phenotypic correlation estimates were obtained using the delta method (Lynch & Walsh, 1997). Matrix computations necessary to obtain the standard errors were performed using SAS Proc IML (SAS Institute Inc., 1985). Approximate 95% confidence intervals were constructed for the genetic variance, heritability, and correlation estimates as the parameter estimate plus or minus twice the approximate standard error (Lynch & Walsh, 1997). Estimates were considered significantly different if their 95% confidence intervals did not overlap.

Evaluation and statistical analysis of fatty acid contents

Seeds for analysis were taken from grain bulked over replications of the experiment grown near Ames, IA in 1992. A single sample of approximately 1 g of groats was analyzed for each line in the study. Fatty acid contents were measured on each genotype by gas chromatography, as described by Schipper et al. (1991). The ratio of unsaturated fatty acid (18:1, 18:2, and 18:3) content to saturated fatty acid (16:0 and 18:0) content (U:S) was computed for each genotype.

One-way analyses of variance were conducted for each trait using SAS Proc GLM (SAS Institute Inc., 1990), with total variation partitioned into differences among cycles and differences among genotypes nested within cycles (representing the residual variance). Checks were included in this analysis as a separate cycle population. Cycle and check group means and standard errors for cycle and check group mean comparisons were computed from this analysis. Checks were then deleted from the data set, and linear regression on number of cycles of selection was performed for each trait using SAS Proc Reg (SAS Institute Inc., 1990). A multiple regression model including linear and quadratic responses over cycles was also performed. If the quadratic term was significant, the linear and quadratic regression estimates from the

Table 1. Mean groat oil content, fatty acid contents, and the ratio of poly- and monounsaturated fatty acid content to saturated fatty acid content (U:S) of oat lines representing the base population (C0), populations resulting from one through nine cycles of recurrent selection for higher oil content (C1-C9), and check cultivars

Population	Groat-oil content*		Fatty acid contents					U:S
	Whole sample	Subsample	16:0	18:0	18:1	18:2	18:3	
C0	9.8	9.4	16.0	2.2	44.1	36.7	1.0	4.5
C1	10.7	10.0	15.6	2.4	45.2	35.8	1.0	4.6
C2	11.3	10.8	15.7	2.3	45.3	35.8	0.9	4.6
C3	12.0	11.4	15.2	2.5	46.0	35.5	0.9	4.7
C4	12.5	12.0	14.9	2.4	46.8	35.1	0.8	4.8
C5	12.8	12.3	15.0	2.6	46.5	35.0	0.8	4.7
C6	13.8	13.1	14.5	2.5	47.4	34.9	0.8	4.9
C7	15.0	14.6	14.3	2.7	49.2	34.6	0.8	4.9
C8	15.1	14.7	13.8	2.6	49.5	32.8	0.8	4.9
C9	15.8	15.2	13.9	2.7	50.2	32.4	0.8	5.1
Checks	7.5	7.1	15.8	1.6	40.2	41.0	1.4	4.7
LSD (0.05) check vs. cycle	1.0	0.9	1.3	0.3	1.4	2.7	0.1	0.3
LSD (0.05) cycle vs. cycle	0.3	0.6	0.8	0.2	0.9	1.7	0.1	0.2

*Groat-oil content mean for each cycle was computed both for the whole sample of 100 lines tested per cycle in three locations, as reported by Frey & Holland (1999) and for the subsample of 20 lines per cycle tested at Ames only, corresponding to the same source of seed used for oil composition analysis in this experiment.

combined model are reported. If the quadratic term was not significant, the linear regression estimate from the reduced linear regression model is reported. Response per cycle for each fatty acid content was reported in the same manner as for agronomic characters. Phenotypic correlations between groat-oil content and the various fatty acid contents were estimated using SAS Proc Corr. In order to test if the phenotypic correlations between the traits changed linearly over cycles, the correlations were estimated for each cycle individually, and for each pair of traits the estimated correlation coefficient was regressed on cycles of selection using SAS Proc Reg.

Table 2. Linear and quadratic regression estimates for response of fatty acid contents and ratio of unsaturated to saturated fatty acid contents (U:S) over cycles of selection

Trait	$\hat{\beta}_l$ %	$\hat{\beta}_q$ $(\%)^2$	Response per cycle %
16:0	–0.24***	NS	–0.24
18:0	0.05***	NS	0.05
18:1	0.39**	0.03*	0.67
18:2	–0.42***	NS	–0.42
18:3	–0.06***	0.004***	–0.02
U:S	0.06***	NS	0.06

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
NS = Not significant at the 0.05 probability level.

Results and discussion

Fatty acid composition

In order to determine if our sample of seed of 20 random lines per cycle grown at Ames was sufficient to represent the trends in groat-oil content of the selection populations, we computed the mean groat-oil content of the subsample of 20 random lines per cycle grown at Ames and compared this to the mean of all 100 lines averaged over three locations (Frey & Holland, 1999). The relative differences among cycle means were consistent between the Ames subsample and the whole sample, with the Ames subsample means being consistently 0.4 to 0.8% lower than the whole sample means (Table 1). These differences are not due to genotypic sampling, because

the cycle means based on 100 lines per cycle from Ames are within 0.2% of the cycle means based on 20 lines per cycle from Ames in all cases except for C8, for which the subsample mean was 0.5% higher than the mean based on 100 lines (data not shown). Therefore, the differences between the subsample oil content means and the whole sample means are primarily due to the environmental effect of the Ames growing location. Frey & Holland (1999) noted that environment effects tended to cause small changes in the groat-oil content cycle means, but did not affect the relative differences among cycle means in this population. Schipper et al. (1991) reported that heritabilities of fatty acid contents were very high, indicating that genotype-by-environment interaction was not substantial in this population. We conclude that our subsamples of entries were representative of their reference populations for oil content and quality.

Significant differences were observed among cycle means for all fatty acid contents and the ratio of unsaturated to saturated fatty acid contents (U:S). Groat oil content increased from 9.8% to 15.8% between C0 and C9 (Table 1; Frey & Holland, 1999). This increase in groat-oil content was accompanied by correlated increases of stearate content from 2.2% to 2.7% and oleate content from 44.1% to 50.2% between C0 and C9 (Table 1). Palmitate content decreased from 16.0% to 13.9%, linoleate content decreased from 36.7% to 32.4%, and linolenate content decreased from 1.0% to 0.8% between C0 and C9 (Table 1). The increase in oleate content more than offset the decreases in linoleate and linolenate contents, resulting in an increase in U:S from 4.5 to 5.1 between C0 and C9. Overall, the dietary quality of oat oil improved as groat-oil content increased due to selection.

The C0 population mean and the mean of the check cultivars differed significantly for all fatty acids except for palmitate, but they did not differ for U:S (Table 1). The differences between the experimental population mean and the check means for groat-oil content and fatty acid contents increased after nine cycles of selection, and differences between the checks and the experimental population in palmitate content and U:S were also significant by C9 (Table 1). Therefore, the oil quality of the C9 population is better than that of standard oat cultivars.

The linear responses of palmitate, stearate, and linoleate contents, and U:S over cycles of selection for higher oil content were significant, with no evidence of quadratic response (Table 2). Thus, it is likely that these traits would continue to respond to selection for higher oil content in this population. There was a significant positive quadratic response in linolenate content (Table 2), indicating that the decrease in linolenate content observed between C0 and C6 by Schipper et al. (1991) did not continue through C9. There were no significant changes in linolenate content between cycles 4 and 9 (Table 1). This result supports the conclusion of Schipper et al. (1991) that genetic variation for linolenate content was exhausted by C6. There was also a significant quadratic response of oleate content over cycles of selection (Table 2). Both linear and quadratic responses of oleate were positive, suggesting that the response in oleate content was increasing in later cycles. Trends of direct and indirect responses of fatty acids and oil content to selection observed in soybean are congruent with our results (Carver et al., 1986; Rebetzke et al., 1998). White (1992) suggested that the primary reason that oleate content tends to increase, while palmitate and linoleate contents tend to decrease as total oil content increases, is that increases in oil content are due to increases in the storage lipid fraction, which is richer in oleate than the structural lipid fraction.

The phenotypic correlation coefficients followed the pattems observed in the responses of the different traits to selection for higher groat-oil content. All of the traits that increased due to selection for higher oil content (stearate content, oleate content, and U:S) were positively correlated with groat-oil content, while all of the traits that decreased due to selection for higher oil content (palmitate, linoleate, and linolenate contents) were negatively correlated with groat-oil content (Table 3). Negative correlations between oil content and palmitate, linoleate, and linolenate contents and positive correlations between oil content and oleate content have been reported consistently in oat (de la Roche et al., 1977; Karow & Forsberg, 1984). Negative correlations between oleate content and palmitate, linoleate, and linolenate contents in oat were also reported by Thro et al. (1985), Forsberg et al. (1974), and Schipper et al. (1991). There were no linear changes in correlations over cycles, indicating that selection altered the levels of the different fatty acids, but not their phenotypic relationships.

The mean oil quality of the C9 population was better than both the C0 population and the check cultivars, with lower palmitate, linoleate and linolenate contents and higher oleate content and ratio of unsaturated to saturated fatty acid contents. The C9 population has good oil quality relative to typical soybean and maize oils, as given by de Man (1990), with much higher

Table 3. Phenotypic correlations among groat-oil content, fatty acid contents, and the ratio of poly- and monounsaturated fatty acid content to saturated fatty acid content (U:S) estimated from all selection populations

	16:0	18:0	18:1	18:2	18:3	U:S
Groat-oil content	–0.49***	0.45***	0.83***	–0.46***	–0.40***	0.42***
16:0	–0.21**	–0.52***	NS	0.29***	–0.84***	
18:0	0.41***	–0.38***	–0.25***	NS		
18:1	–0.58***	–0.45***	0.49***			
18:2	0.17*	NS				
18:3	–0.28***					

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
NS = Not significant at the 0.05 probability level.

Table 4. Means of CO through C9 populations and checks for test weight, 100-seed weight, biomass, straw yield, harvest index, averaged over three locations and height, and heading date measured at one location

Population	Test weight g m^{-3}	100-seed weight g	Biomass g m^{-2}	Straw yield g m^{-2}	Harvest index %	Height m	Heading date dapt*
C0	408	3.33	811	446	45.0	0.76	79.3
C1	405	3.19	819	454	44.6	0.76	79.0
C2	403	3.29	783	429	45.2	0.76	78.8
C3	403	3.26	759	415	45.3	0.76	78.2
C4	391	3.04	732	400	45.4	0.75	78.3
C5	388	3.00	706	382	45.9	0.75	77.6
C6	382	2.90	697	378	45.9	0.74	78.1
C7	372	2.75	546	300	44.9	0.70	79.6
C8	387	2.80	633	348	44.9	0.73	78.9
C9	402	2.67	636	350	44.7	0.74	78.4
Checks	432	3.53	767	411	46.2	0.76	80.2
LSD (0.05) cycle vs cycle	10	0.18	31	19	NS	0.01	0.7
LSD (0.05) cycle vs check	20	0.28	81	46	NS	0.03	2.0

* Days after planting.
NS = no significant differences among cycles, based on F-test.

Table 5. Linear (β_l) and quadratic (β_q) regression coefficient estimates (and their standard errors) and mean response of grain quality and agronomic traits to nine cycles of recurrent selection for higher oil content

Parameter estimate	Test weight g m^{-3}	100-seed weight g	Biomass g m^{-2}	Straw yield g m^{-2}	Height m	Heading date dapt*
$\hat{\beta}_l$	–7.3 (0.6)	–0.071 (0.003)	–25.9 (0.9)	–19.3 (1.9)	–0.48 (0.05)	–0.42 (0.09)
$\hat{\beta}_q$	0.47 (0.07)	NS	NS	0.6 (0.2)	NS	0.043 (0.009)
Mean response per cycle	–0.7	–0.071	–25.9	–10.7	–0.48	–0.1

* Days after planting.

Table 6. Genotypic variance and heritability estimates (and their standard errors) of grain quality and agronomic traits of C0 through C9 populations

Cycle	Test weight		100-seed weight		Straw yield		Biomass		Harvest index		Heading date		Height	
	$\hat{\sigma}^2_G$	$\hat{H}$	$\hat{\sigma}^2_G$	$\hat{H}$	$\hat{\sigma}^2_G$	$\hat{H}$	$\hat{\sigma}^2_G$	$\hat{H}$	$\hat{\sigma}^2_G$	$\hat{H}$	$\hat{\sigma}^2_G$	$\hat{H}$	$\hat{\sigma}^2_G$	$\hat{H}$
	$(kg\ m^{-3})^2$		$(g)^2$		$(g\ m^{-2})^2$		$(g\ m^{-2})^2$		$(\%)^2$		$(d)^2$		$(m)^2$	
0	436	0.68	0.096	0.64	3048	0.28	7834	0.26	4.7	0.20	3.8	0.50	0.0015	0.47
	(73)	(0.04)	(0.016)	(0.05)	(666)	(0.05)	(1784)	(0.05)	(1.3)	(0.05)	(0.9)	(0.08)	(0.0003)	(0.08)
1	265	0.57	0.047	0.40	1573	0.16	4616	0.16	1.5	0.06	3.6	0.46	0.0006	0.27
	(48)	(0.05)	(0.010)	(0.06)	(473)	(0.04)	(1254)	(0.04)	(0.9)	(0.04)	(0.9)	(0.08)	(0.0002)	(0.09)
2	298	0.58	0.057	0.46	1265	0.13	3831	0.14	2.9	0.12	3.7	0.48	0.0013	0.45
	(54)	(0.05)	(0.011)	(0.06)	(398)	(0.04)	(1190)	(0.04)	(1.0)	(0.04)	(0.9)	(0.08)	(0.0003)	(0.08)
3	150	0.45	0.061	0.54	2236	0.20	5518	0.20	2.8	0.10	5.3	0.57	0.0011	0.34
	(31)	(0.05)	(0.011)	(0.06)	(573)	(0.05)	(1400)	(0.04)	(1.1)	(0.04)	(1.1)	(0.07)	(0.0003)	(0.09)
4	302	0.63	0.045	0.38	918	0.13	3110	0.15	1.7	0.08	1.6	0.25	0.0012	0.37
	(52)	(0.05)	(0.011)	(0.07)	(314)	(0.04)	(962)	(0.04)	(0.8)	(0.04)	(0.7)	(0.09)	(0.0004)	(0.09)
5	345	0.64	0.045	0.46	1346	0.17	4139	0.18	3.0	0.13	6.6	0.65	0.0014	0.38
	(59)	(0.05)	(0.009)	(0.06)	(373)	(0.04)	(1.67)	(0.04)	(I.0)	(0.04)	(1.2)	(0.06)	(0.0004)	(0.09)
6	342	0.68	0.053	0.66	810	0.11	1432	0.07	3.8	0.17	4.7	0.62	0.0011	0.37
	(57)	(0.04)	(0.009)	(0.05)	(300)	(0.04)	(708)	(0.04)	(I.0)	(0.04)	(0.9)	(0.06)	(0.0003)	(0.09)
7	592	0.70	0.069	0.60	2829	0.26	10530	0.32	10.4	0.29	8.7	0.69	0.0030	0.51
	(116)	(0.06)	(0.013)	(0.06)	(663)	(0.05)	(2213)	(0.05)	(2.2)	(0.05)	(1.6)	(0.05)	(0.0007)	(0.08)
8	800	0.81	0.104	0.77	2765	0.25	9348	0.28	3.8	0.13	5.7	0.64	0.0017	0.44
	(137)	(0.04)	(0.019	(0.04)	(620)	(0.05)	(1978)	(0.05)	(1.2)	(0.04)	(1.1)	(0.06)	(0.0004)	(0.08)
9	798	0.85	0.056	0.48	4609	0.42	17856	0.50	6.2	0.20	5.5	0.65	0.0016	0.44
	(144)	(0.03)	(0.023)	(0.15)	(836)	(0.05)	(3028)	(0.05)	(1.6)	(0.04)	(1.0)	(0.06)	(0.0004)	(0.08)

oleate content than both soybean and maize and much lower linolenate content than soybean, but the palmitate content of the oat C9 population is still higher than maize or soybean oil. Continued reduction in palmitate content of oat oil would be desirable. In summary, the changes in fatty acid contents that have occurred as correlated responses to selection for high oil content have been favorable, leading to improved oil quality as well as increased oil content and oil yield.

Grain guality and agronomic characteristics

Significant differences were observed among cycle means for all traits except harvest index (Table 4). Later cycles of selection tended to have lower test weight, seed weight, straw yield, biomass, and height (Table 4). Cycle 7 had the lowest mean test weight, biomass, straw yield and height (Table 4) and Frey & Holland (1999) reported that C7 also had the lowest grain yield and second lowest oil yield among all cycles. Test weight, biomass, straw yield, and height increased from C7 to C9 (Table 4), as did grain yield (Frey & Holland, 1999). These trends were reflected in the significant quadratic regression responses observed for test weight and straw yield (Table 5). In both cases, the quadratic regression parameter estimate was positive while the linear regression estimate was negative, indicating that the negative trends observed in earlier cycles were significantly reversed in later cycles. For all traits measured, except harvest index, negative linear and mean responses over cycles of selection were observed (Table 5). Thus, in general, the grain quality and agronomic performance of oat lines with higher oil contents were lower than the base population and the checks. The negative responses observed for these traits confirm those reported by Frey & Schipper (1992) and indicate that they have continued beyond C6. The observed increases in test weight in combination with low seed weight observed in the last two cycles suggest that the proportion of thin 'pin' oats may have increased in these late cycles. Pin oats are very light, but tend to have high test weights because they can pack tightly into containers used to measure test weight (Burnette et al., 1992).

Significant genetic variance was observed for all traits in all cycles (Table 6). Without exception, ge-

netic variances and heritabilities decreased from C0 to C1, but the decreases were significant only for seed weight. This trend was also observed by Branson & Frey (1989a) and Schipper & Frey (1992b). Hallauer & Miranda (1988) suggested that in numerous maize (*Zea mays* L.) recurrent selection studies genetic variance for the selected trait decreased after the first cycle of selection but remained relatively constant thereafter. Although genetic variances generally decreased after the first cycle of selection in this population, they tended to increase in later cycles to equal or exceed the original variances. Heritability for test weight and biomass and genetic variance for biomass were significantly greater in C9 than C0. These results are congruent with those of Frey & Holland (1999), who found no evidence for decreased genetic variance for groat-oil content, grain yield, groat fraction, or oil yield. We suggest that long-term selection for quantitative traits in broad-based oat populations is likely to be effective at making significant changes in trait means without reducing genetic variances. Long-term selection studies for oil content in maize have also shown that selection in broad-based populations can effect very large changes in the mean oil content of the population without reducing genetic variance if large effective population sizes are maintained to prevent genetic drift (Dudley, 1977; Miller et al., 1981; Misevic & Alexander, 1989). Bulmer (1985) predicted that selection will decrease genetic variance for the selected trait by increasing gametic phase disequilibrium among alleles affecting the selected trait, and this could also affect unselected traits if they were correlated with the selected trait. Recombination associated with repeated intermatings in the recurrent selection scheme, however, may reduce gametic disequilibrium, and this may result in increased genetic variance.

The genotypic correlations between oil content and the grain quality and agronomic traits were largely negative or not significant (Table 7), with the exception of heading date. Test weight and oil content tended not to be significantly correlated, except in C9, in which they were significantly positively correlated. This may be a result of a general change in kernel shape in the higher oil genotypes in the last cycles. If higher oil oats tended to have thinner kernels in the last cycle of selection, this may explain the positive correlation in that cycle. This is supported by the negative correlations between seed weight and oil content, which were consistently negative over cycles, but were very strongly negative (r_g = –0.91) in the last cycle. Direct selection for improved grain type in

Table 7. Genotypic correlations (and the lower and upper bounds of their approximate 95% confidence intervals) between groat-oil content and grain quality and agronomic traits of C0 through C9 populations

Cycle	Test weight	100-seed weight	Straw yield	Biomass	Harvest index	Heading date	Height
0	–0.17 (–0.39, 0.05)	–0.27 (–0.49, 0.05)	–0.12 (–0.38, –0.14)	–0.18 (–0.44, 0.08)	–0.10 (–0.38, 0.18)	–0.10 (–0.36, 0.16)	0.14 (–0.12, 0.40)
1	–0.16 (–0.40, 0.08)	–0.43 (–0.65, –0.21)	0.00 (–0.30, 0.30)	–0.08 (–0.38, 0.22)	–0.43 (–0.95, 0.09)	–0.11 (–0.37, 0.15)	–0.03 (–0.29, 0.35)
2	0.01 (–0.23, 0.25)	–0.23 (–0.47, 0.01)	–0.34 (–0.64, –0.04)	–0.33(–0.63, –0.03)	0.09 (–0.24, 0.42)	0.01 (–0.25, 0.27)	–0.10 (–0.38, 0.18)
3	–0.31 (–0.55, 0.07)	–0.21 (–0.45, 0.03)	0.20 (–0.08, 0.48)	0.14 (–0.14, 0.42)	–0.21 (–0.53, 0.11)	0.19 (–0.05, 0.43)	0.18 (–0.12, 0.48)
4	–0.11 (–0.35, 0.13)	–0.20 (–0;48, 0.08)	–0.16 (–0.50, 0.18)	–0.07 (–0.39, 0.25)	0.32 (–0.18, 0.82)	0.00 (–0.41, 0.41)	0.01 (–0.33, 0.35)
5	–0.10 (–0.38, 0.08)	–0.21 (–0.49, 0.07)	0.27 (–0.03, 0.57)	0.30 (0.00, 0.60)	0.07 (–0.28, 0.42)	0.39 (2.12, 0.65)	0.20 (–0.16, 0.56)
6	–0.17 (–0.41, 0.07)	–0.27 (–0.51, 0.03)	0.24 (–0.14, 0.62)	0.37 (–0.07, 0.81)	0.04 (–0.28, 0.36)	0.67 (0.47, 0.87)	0.22 (–0.12, 0.56)
7	–0.02 (–0.28, 0.24)	–0.32 (–0.56, –0.08)	–0.13 (–0.41, 0.15)	–0.06 (–0.32, 0.20)	0.32 (0.08, 0.56)	0.33 (0.13, 0.53)	0.08 (–0.20, 0.36)
8	0.01 (–0.17, 0.19)	–0.65 (–0.83, –0.47)	–0.04 (–0.32, 0.24)	–0.09 (–0.37, 0.19)	–0.16 (–0.47, 0.17)	0.36 (0.06, 0.66)	0.08 (–0.26, 0.42)
9	0.37 (0.15, 0.59)	–0.91 (–1.00, –0.79)	–0.25 (–0.49, –0.01)	–0.28 (–0.50, –.06)	–0.23 (–0.51, 0.05)	0.37 (0.13, 0.61)	–0.22 (–0.04, 0.43)

these higher oil genotypes may be necessary. The significantly positive correlations between heading date and oil content are surprising because mean heading dates decreased whereas groat-oil content increased over cycles. Culling of the experimental lines for general adaptation to Iowa growing environments before selection for oil content may have been the primary cause of the tendency toward earlier heading in the later cycles.

The negative genetic correlations between groat-oil content and agronomic characters, particularly in the C9 population (Table 7), and the deterioration of mean grain quality and agronomic trait performance over cycles of selection (Tables 4 and 5) indicate that the development of high-oil oat cultivars will require more than just selection for high oil content. The use of an optimal selection index to improve both groat-oil content and grain quality and agronomic characteristics is recommended. Simultaneous improvement of multiple traits, some of which are negatively genetically correlated, is most efficiently achieved through the use of an optimal selection index (Baker, 1986). The effectiveness of a selection index depends on good estimates of genotypic and phenotypic covariances among the traits to be included in the index, however. The approximate 95% confidence intervals of genotypic correlation estimates tended to be large, and overlapped zero in many cases (Table 7), suggesting that the sample sizes used in this study were not sufficiently large to obtain precise estimates of the covariance components. Population sample sizes were greater in the actual selection phases of the recurrent selection experiment, so this requirement should not be a practical hindrance. A change in the recurrent selection scheme would be required, however, to conduct replicated line evaluations rather than individual plant evaluations as was done in the recurrent phenotypic selection method. Replication of lines would be necessary to estimate the genotypic components of variance and covariance required for the use of a selection index.

In summary, selection for increased groat-oil content improved oil quality but reduced grain quality and agronomic performance of the population. The maintenance of genetic variances for grain quality and agronomic traits in this population suggests that selection for agronomically adapted genotypes within the later cycles of selection should be possible. Because of the negative genetic correlations between oil content and grain quality and agronomic traits, multi-trait index selection within the later cycles would be the most efficient method to combine improvements in oil content and agronomic performance.

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